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Modulation of fish fibroblast proliferation with β -glucan and hydrogen peroxide during scratch-wound healing *in vitro*.

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Regulation of cellular proliferation is an essential phase of wound healing to reach tissue homeostasis and complete repair. Although several diseases and even mechanical injury can damage fish tissues, only a few studies have been directed to tissue regeneration and modulation of cell proliferation during wound healing in fish. *In vivo* studies in cyprinids have demonstrated the capacity of β -glucan and hydrogen peroxide to modulate cellular proliferation during wound healing. However, the mechanisms through which the modulation is achieved are remains to be understood. Fibroblasts are responsible of collagen deposition and synthesis of extracellular matrix components and have shown to play a key role in the outcome of wound healing.

This study analyses the modulation ability of β -glucan and hydrogen peroxide on carp fibroblasts *in vitro*. Using a practical and convenient PhotoID proliferation assay, which employs microscopy pictures and statistical image analysis, the wound area recovery in scratch-wounded CCB fibroblast cell cultures was calculated after stimulation with β -glucan (MacroGard[®]) and hydrogen peroxide. Fibroblast stimulation with 100 μ g/ml of β -glucan showed a slight increase in percentage of wound recovery after 12 hours of stimulation, confirming the modulatory capacity of β -glucan during cellular proliferation. Later measurements did not show significant differences, suggesting that repeated doses of β -glucan might be required to achieve maximum modulator ability. Furthermore, fibroblast stimulation with 3-5 μ M of hydrogen peroxide moderately increased the percentage of wound recovery, 25-100 μ M impaired fibroblasts proliferation and higher doses of hydrogen peroxide caused cell death. The later results show the ability of oxygen radicals such as hydrogen peroxide to drive the fate of tissue regeneration through the establishment of environments suitable for tissue regeneration (3-5 μ M), pathogen eradication (25-100 μ M) or oxidative stress (<100 μ M).